Description

Container for Immunologic Assay

Technical Field

The present invention relates to a container used for storage, dilution, or reaction of a reagent and/or a test sample, in an immunoassay for detecting an antigen or an antibody through antigen-antibody reaction.

Background Art

Conventional immunoassays employ a polystyrene- or polypropylene-made container for storage and dilution of a reagent or a sample which is to be used. However, molecules contained in the reagent or the sample are non-specifically adsorbed onto such a container, and such adsorption necessarily causes loss of the reagent or the sample, as well as variation in concentration of a solution containing the reagent or the sample.

In recent years, in accordance with diversification of immunoassay methods, in most cases, naturally occurring substances have been used after extraction and purification, particularly in the drug production department of drug manufacturers. Generally speaking, such substances are obtained in very small amounts and thus are quite expensive. Therefore, reduction in the amount of substance during storage or dilution, which is caused by physical adsorption

onto a container, is not negligible.

When samples used for clinical diagnosis, such as serum and urine, are collected from patients, placed in a container, and stored therein until the samples are subjected to assay, clinically important proteins contained in the samples, such as albumin, transferrin, and immunoglobulin, are adsorbed onto the container. Most containers used for clinical diagnosis, including syringes and cups used in the step of collecting a sample, tubes used in the step of storing the sample, and centrifugation tubes and test tubes used in the step of purifying, concentrating, or diluting the sample, are formed from polypropylene or polystyrene, and such a container is not subjected to surface treatment. Therefore, when even a trace amount of proteins contained in the sample is adsorbed onto the container in each step, the concentration of the proteins is expected to vary greatly after all the steps have been performed, as compared with the concentration of the proteins at the time of collection of the sample.

In general, the price of a reagent in immobilized form accounts for about 80% the cost of a clinical test kit sold by a clinical test drug manufacturer. Therefore, when reduction in the reagent due to adsorption onto a container is suppressed, production costs are greatly reduced.

In a solid phase method (a type of immunoassay method), assay is carried out by utilizing proteins immobilized onto the surface of a container for an immunoassay. Therefore, a

solid phase method employs a container subjected to "high adsorption treatment," in which, in order to increase the amount of a reagent which is to be immobilized onto the surface of the container, a hydrophilic-hydrophobic balance of the surface is regulated through introduction of a functional group such as a hydroxyl group, thereby increasing the saturation adsorption amount of the reagent.

In recent years, in order to shorten immunoassay time and to carry out immunoassay on a large scale, immunoassay methods making use of an automatic analyzer (robot) have been developed. Such methods have rapidly become prevalent, particularly in the drug production department of drug manufacturers.

When an assay is carried out by means of a conventional solid phase method, a washing step for eliminating non-immobilized excess molecules is required. However, an automatic analyzer encounters difficulty in carrying out the washing step, in which fractional injection and suction of a washing solution are repeated. Therefore, a sequential addition method has been under development as an immunoassay method suitable for an automatic analyzer, because such a method does not require separation of a reacted substance and a non-reacted substance through a washing step.

In a sequential addition method, immobilization of molecules is not carried out during reaction, and reaction is carried out in a solution. Therefore, when a container having a surface subjected to the aforementioned high

adsorption treatment is used, unwanted adsorption of the molecules impedes reaction in the solution or lowers the reaction efficiency.

In recent years, in accordance with progress in measurement techniques, evaluations through a fluorescence method or an emission method have been established, the method having high sensitivity as compared with absorbance assay by means of a conventional colorimetric method. Therefore, in the future, unwanted adsorption of molecules onto a container is expected to induce problems in such an assay method having high sensitivity.

At the present time, a container used for such a method is provided without consideration of molecular adsorption; i.e., the container is formed from polystyrene or polypropylene in consideration of only shapability, transparency, and low-temperature resistance, and the container is not subjected to surface treatment for suppressing adsorption of molecules. From the viewpoint of characteristics of the container, no attempt has been made to solve problems such as loss of a reagent and reduction in sensitivity.

However, in order to control non-specific adsorption of molecules onto the surface of a container for immunoassays, several techniques have hitherto been studied and carried out.

For example, a blocking method is most widely carried out, in which a container is coated with a protein inactive to a sample which is to be assayed. Since the method

basically utilizes non-specific adsorption of the protein onto the container, blocking effects may differ from container to container, and may depend on the state of the protein. In addition, since the inactive protein is non-specifically adsorbed onto the container, the protein is easily detached from the container into a solution, and thus the container cannot be used for storing the solution. Japanese Patent Application Laid-Open (*kokai*) Nos. 6-174726 and 7-128336 disclose a technique in which such detachment of a protein is eliminated by chemically immobilizing the protein onto a container. However, the structure of the protein may vary in accordance with drying temperature, storage temperature, and storage time, and thus the container is not widely used in practice.

When the higher-order structure of a protein adsorbed onto a container varies, the protein induces secondary adsorption. When a protein which is inactive in a free state is adsorbed onto or chemically bound to a container, the protein cannot completely maintain its inactive state, due to alteration of the higher-order structure. Therefore, even when adsorption of another protein onto the container can be prevented, variance of the higher-order structure induces secondary adsorption between the proteins.

Secondary adsorption between proteins varies with types of proteins, and thus a protein suitable for blocking must be chosen every time a sample which is to be assayed is changed. When a solution containing different proteins, such as serum,

is used as a sample, no blocking protein can control adsorption of all the proteins contained in the sample.

Disclosure of the Invention

In view of the foregoing, the present inventors have performed extensive studies on characteristics of a container, and have found that when the saturation amount of molecules which are adsorbed onto the container, the molecules being used for an immunoassay, is controlled to a predetermined value or less, loss of a reagent or a sample is prevented during storage, dilution, and reaction, and the sample can be assayed at high sensitivity. The present invention has been accomplished on the basis of this finding.

Accordingly, the present invention provides a container for an immunoassay in which the saturation adsorption amount of molecules used for the assay is 1×10^{-1} pmol/cm^2 or less.

Brief Description of Drawings

Fig. 1 shows the concentration of proteins after bovine serum and albumin have been stored in the container for an immunoassay of the present invention at -80°C for 48 hours. Fig. 2 shows reaction efficiency when an immunoassay is carried out in the container of the present invention.

Best Mode for Carrying Out the Invention

In a conventional polystyrene- or polypropylene-made container for an immunoassay, the adsorption amount of

molecules (e.g., proteins) is about 1-10 pmol/cm^2 or more; i.e., about 20-50% of molecules (e.g., proteins) used for an immunoassay are adsorbed onto the container, although the adsorption amount varies in accordance with the concentration of a solution containing such molecules and the contact area between the molecules and the container. When the adsorbed molecules (20-50% of all the molecules) are essential for reaction in the solution, reaction efficiency; i.e., assay sensitivity, is reduced by 20-50%. Meanwhile, when the adsorbed substance is such that it undergoes molecular structural changes due to adsorption to thereby cause unwanted reaction, considerable noise would result.

Therefore, a container onto which no molecules used for an immunoassay are adsorbed is most ideal, but when the adsorption amount of molecules is substantially reduced to 1/10-1/100 with respect to the current level, satisfactory effects will be obtained.

Although the adsorption amount of molecules contained in a solution varies with the identity of the molecules, temperature, concentration of the solution, and the pH of the solvent, the container desirably meets the following conditions: the saturation adsorption amount of the molecules used in the immunoassay is 1×10^{-1} pmol/cm^2 or less under the specific conditions——in terms of concentration of the solution, temperature, and pH of the solvent——under which the reaction and assay are carried out. In the case in which serum is used for an immunoassay, since serum is usually

diluted up to 1/10, the effect of the invention can be attained if the saturation adsorption amount of the molecules which participate in and/or affect the assay, among all molecules contained in the diluted serum, is always 1×10^{-1} pmol/cm^2 or less at the diluted concentration of serum and under the specific conditions——in terms of concentration of the solution, temperature, and pH of the solvent——under which the reaction and assay are carried out.

Similarly, when the container is used for storage and dilution of a reagent, the effect of the invention can be attained if the saturation adsorption amount of the molecules that undergo storage and dilution is always 1×10^{-1} pmol/cm^2 or less under the specific conditions——in terms of concentration of the solution, temperature, and pH of the solvent——under which the reagent is removed from the storage container or dilution is carried out. In many cases, the reagent is stored in the container at a temperature as low as -80°C. However, adsorption of the molecules is an equilibrium reaction, and thus, it would be sufficient if the saturation adsorption amount of molecules is 1×10^{-1} pmol/cm^2 or less under the specific conditions——in terms of concentration, temperature, and pH——under which the reagent is removed from the container.

The saturation adsorption amount of the molecules is more preferably 1×10^{-2} pmol/cm^2 or less, much more preferably 1×10^{-3} pmol/cm^2 or less.

Examples of the molecules used in an immunoassay

include proteins (e.g., enzymes, physiologically active proteins, and antibodies), nucleic acids, and physiologically active substances. Of these, proteins are particularly preferable. The saturation adsorption amount of the molecules can be measured by means of colloidal gold labeling immunoassay.

In the point that adsorption of a protein is prevented, the present invention exerts excellent effects in addition to the aforementioned characteristic feature. Usually, when a protein is adsorbed onto a container, the structure of the protein is varied. Therefore, when an immunoassay is carried out, although a target protein is contained in a sample to be assayed, the protein may fail to be detected by an antibody, due to variation in the structure of the protein. When a clinical test is carried out, in practice serum whose structure has been altered due to adsorption is assayed, even though serum must be assayed in the same state in which the serum is present in an organism. According to the present invention, since a protein is not adsorbed onto the container, the structure of the protein is not altered, and thus when a clinical test is carried out by use of the container, serum can be assayed in a state similar to that in which serum is present in an organism. Therefore, the container of the present invention is very advantageously used as a container for an immunoassay.

In a container for an immunoassay, the saturation adsorption amount of molecules must be decreased at a portion

with which a reagent or a sample is brought into contact; specifically, an inner surface of the container. Therefore, the molecular saturation adsorption amount at an inner surface of the container should be at least 1×10^{-1} pmol/cm^2 or less.

In order to decrease the saturation adsorption amount of molecules at an inner surface of the container to 1×10^{-1} pmol/cm^2 or less, preferably, at least the inner surface is formed from a highly hydrophilic polymer or a highly hydrophobic polymer, or is coated with a highly hydrophilic polymer or a highly hydrophobic polymer. More preferably, at least the inner surface is coated with a highly hydrophilic polymer or a highly hydrophobic polymer. Much more preferably, at least the inner surface is coated with a highly hydrophilic polymer. Particularly preferably, at least the inner surface is coated with an ultra-hydrophilic polymer.

Examples of highly hydrophobic polymers include fluorine-containing resins such as polytetrafluoroethylene (PTFE) and silicon-containing resins. When the surface of the container is coated with a hydrophobic polymer, the surface may be coated with the aforementioned hydrophobic polymer, or the container may be fluorinated, thereby forming a fluorinated polymer film on the surface thereof.

No particular limitation is imposed on the highly hydrophilic polymer, so long as the polymer contains a hydrophilic group such as a carboxyl group or a hydroxyl

group. Examples of such a hydrophilic polymer include polymethacrylic acid, (meth)methacrylic acid-alkyl methacrylate copolymers, polyhydroxyalkyl methacrylates (e.g., polyhydroxyethyl methacrylate), hydroxyalkyl methacrylate-alkyl methacrylate copolymers, polyoxyalkylene-group-containing methacrylate polymer and copolymers containing the polymer, polyvinyl pyrrolidone, ethylene-vinyl alcohol copolymers, (2-methacryloyloxyethylphosphocholine) polymers (MPC) and copolymers containing the polymers (Seitai Zairyo, Vol. 9, No. 6, 1991), and phospholipid·polymer composites (Japanese Patent Application Laid-Open (*kokai*) Nos. 5-161491 and 6-46831). The container may be formed from such a hydrophilic polymer, or coated with the polymer.

After the container is formed from a suitable material, such as polystyrene, a hydroxyl group or a carboxyl group may be introduced into the surface of the container, to thereby impart high hydrophilicity; i.e., low adsorbability, to the surface of the container. A surface exhibiting low adsorbability can be realized by means of surface modification. For example, when, in consideration of formability, the container is formed from a material which tends to induce non-specific adsorption, such as polystyrene or polypropylene, a carboxyl group, a carbonyl group, and/or a hydroxyl group may be introduced into the surface of the container through plasma exposure, to thereby impart low adsorbability to the surface. When, in consideration of transparency, the container is formed from polymethyl

methacrylate, a carboxyl group may be introduced into the surface of the container through partial hydrolysis of the surface by use of an alkali, to thereby impart low adsorbability to the surface.

When hydrophilicity is imparted to the inner surface of the container by use of a hydrophilic polymer, to thereby reduce the adsorption amount of molecules, the contact angle between the surface and water is preferably 30° or less (highly hydrophilic), more preferably 15° or less, much more preferably 1° or less (ultra-hydrophilic).

When, among the aforementioned hydrophilic polymers, there is employed a polyhydroxyalkyl methacrylate, a polyoxy(C_2-C_4 alkylene-group-containing methacrylate) polymer or a copolymer containing the polymer; a (2-methacryloyloxyethylphosphocholine) polymer or a copolymer containing the polymer; a phospholipid·polymer composite; or polyvinyl pyrrolidone, the contact angle between the surface of the resultant container and water becomes 1° or less (i.e., the container is ultra-hydrophilic), and the saturation adsorption amount of proteins becomes 1×10^{-3} pmol/cm^2 or less, which is particularly preferable.

The product form of the container of the present invention is not particularly limited, and the container may assume conventionally used product forms, including a sample tube, a centrifugation tube, a multi-well plate, and a cuvette. However, in order to carry out storage, dilution, reaction, and assay of a sample in one container, the

container preferably assumes a form of multi-well plate.

Examples

The present invention will next be described in more detail by way of Examples, which should not be construed as limiting the invention thereto.

(Example 1)

A commercially available polypropylene-made 96-well plate (MS-3396P, product of Sumitomo Bakelite Co., Ltd.) was subjected to γ-ray treatment at 70 kGy, to thereby generate a hydroxyl group on the surface of the plate. In the resultant plate, the saturation adsorption amount of proteins was 4.6×10^{-2} pmol/cm^2, and the contact angle between the surface and the water was 27°.

(Example 2)

A commercially available polypropylene-made 96-well plate (MS-3396P, product of Sumitomo Bakelite Co., Ltd.) was coated with a commercially available fluorine-containing coating agent (Scotchguard, product of Sumitomo 3M Ltd.). In the resultant plate, the saturation adsorption amount of proteins was 2.7×10^{-2} pmol/cm^2, and the contact angle between the surface and the water was 126°.

(Comparative Example 1)

A commercially available polypropylene-made 96-well plate (MS-3396P, product of Sumitomo Bakelite Co., Ltd.) was used as a comparative plate. In the plate, the saturation adsorption amount of proteins was 3.7 pmol/cm^2, and the

contact angle between the surface and the water was 92°.

(Comparison of protein recovery percentage in containers usable as storage containers)

For comparison of non-specific adsorption, solutions of an enzyme-labeled anti-bovine-albumin antibody (product of Cosmo Bio) were prepared (concentration of the antibody: 0.1 ng/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL, respectively); each solution was injected into 24 wells of each of the plates of Examples 1 and 2 and Comparative Example 1; the plate was stored at -80°C for 48 hours; and after storage time had elapsed, the concentration of the protein in each solution was measured by use of a substrate solution.

The results are shown in Fig. 1. The results show that the protein recovery percentage is high in the plates of Examples 1 and 2, as compared with the case of the plate of Comparative Example 1.

(Comparison of "in solution" reaction efficiency)

In order to evaluate the efficiency of a reaction in a solution, the following test was carried out by using the plates of Examples 1 and 2 and Comparative Example 1 as reaction containers.

Rat albumin (product of Cosmo Bio) was dissolved in a phosphate buffer (Dulbecco PBS pH 7.4) to thereby prepare solutions (concentration of the albumin: 10 ng/mL, 1 ng/mL, and 0.1 ng/mL, respectively), and each solution was injected into four lines (i.e., 32 wells) (100 µl per well) of each of the plates of Examples 1 and 2 and Comparative Example 1.

Subsequently, a phosphate buffer (Dulbecco PBS pH 7.4) solution of a peroxidase-labeled anti-rat-albumin antibody (product of Cosmo Bio) (concentration of the antibody: 100 ng/mL, respectively) was injected into all the wells (100 µl per well) of each plate.

After reaction had been carried out in each well at 37°C for 30 minutes, the solution in each well was transferred into a 96-well plate for ELISA in which an anti-rat-albumin antibody had been immobilized onto each well in advance, and then reaction was carried out again in each well at 37°C for 30 minutes.

After reaction was completed, a non-reacted peroxydase-labeled anti-rat-albumin antibody was washed with a washing solution (Dulbecco PBS pH 7.4 + 0.05% Tween 20). Subsequently, each plate was allowed to develop color by use of a commercially available chromophoric kit for peroxidase (ML-1120T, product of Sumitomo Bakelite Co., Ltd.), and then absorbance at 450 nm was measured using a plate reader.

The results are shown in Fig. 2. The results show that, in relation to the plate of Comparative Example 1, the absorbance is low when the concentration of the albumin is low; i.e., the reaction in the solution is impeded due to adsorption, and that, in relation to the plates of Examples 1 and 2, linearity is obtained between the concentration of the albumin and the absorbance when the albumin concentration is low; i.e., the antigen-antibody reaction in the solution is efficiently carried out.

(Example 3)

A commercially available polystyrene-made tube (Eiken tube for RIA No. 3, 70-12458) was coated with polyhydroxyethyl methacrylate (P-3932, product of SIGMA). In the resultant tube, the saturation adsorption amount of proteins was 9.1×10^{-4} pmol/cm^2, and the contact angle between the surface and the water was 0°.

(Example 4)

Polytetrafluoroethylene was formed into a tube of the same inner diameter and volume as the tube of Example 1. In the resultant tube, the saturation adsorption amount of proteins was 7.2×10^{-3} pmol/cm^2, and the contact angle between the surface and the water was 126°.

(Comparative Example 2)

A commercially available polystyrene-made tube (Eiken tube for RIA No. 3, 70-12458) was used as a comparative tube. In the tube, the saturation adsorption amount of proteins was 8.1 pmol/cm^2, and the contact angle between the surface and the water was 85°.

(Comparison of assay sensitivity)

In order to evaluate the assay sensitivity of a reaction in a solution, the following test was carried out by use of the tubes of Examples 3 and 4 and Comparative Example 2 as reaction containers and an ELISA ball as a carrier for reaction.

Phosphate buffer (pH 7.4) solutions of biotin hydrazide (product of Dojindo) were prepared in advance (concentration

of biotin hydrazide: 0.125 μg/mL, 0.250 μg/mL, and 0.500 μg/mL, respectively). By use of the solutions, biotin hydrazide was immobilized onto ELISA balls (amino-group-containing ball, product of Sumitomo Bakelite Co., Ltd.) through covalent bonding via glutaraldehyde, to thereby prepare ELISA balls having three different immobilization densities of biotin hydrazide.

A portion of each ELISA ball at which biotin hydrazide was not immobilized was subjected to blocking by use of skim milk so as to prevent adsorption.

Each of the above-prepared ELISA balls was placed into each of the tubes of Example 3, Example 4, and Comparative Example 2 (three tubes for each Example), a phosphate buffer (pH 7.4) solution of peroxydase-labeled avidin (product of Cappel) (concentration of avidin: 1 μg/mL) was injected into each tube (500 mL per tube), and reaction was carried out in each tube at room temperature for 30 minutes.

After the reaction was completed, non-reacted peroxydase-labeled avidin was washed with a washing solution (phosphate buffer pH 7.4 + 0.05% Tween 20). Subsequently, each ELISA ball was allowed to develop color by use of a commercially available chromophoric kit for peroxidase (ML-1120T, product of Sumitomo Bakelite Co., Ltd.), and then absorbance at 450 nm was measured by using a plate reader.

The results are shown in Table 1. The results show that, in Examples 3 and 4, the absorbance varies linearly with respect to the density of biotin hydrazide introduced onto

the surface of the ELISA ball, and that, in Comparative Example 2, the absorbance does not vary with the different densities of biotin hydrazide.

In Examples 3 and 4, peroxydase-labeled avidin is reacted with only biotin hydrazide introduced onto the surface of the ELISA ball, and thus the absorbance is proportional to the density of biotin hydrazide. In contrast, in Comparative Example 2, peroxydase-labeled avidin remains in the tube due to adsorption, and the remaining avidin may act as a background, to thereby reduce assay sensitivity.

Table 1

Biotin-avidin reaction by use of ELISA ball

μg/mL	Example 3	Example 4	Comparative Example 2
0.125	0.2	0.27	1.05
0.25	0.54	0.62	1.03
0.5	0.97	0.91	1.12

(Comparison of protein recovery percentage in containers usable as storage containers)

For comparison of non-specific adsorption, solutions of an enzyme-labeled anti-bovine-albumin antibody (product of Cosmo Bio) were prepared (concentration of the antibody: 0.1 ng/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL, respectively); each solution was injected into 24 wells of each of the plate of Example 3, the plate of Example 4, and the plate of Comparative Example 2; the plates were stored at -80°C for 48 hours; and after storage time had elapsed, the concentration of the protein in each solution was measured by use of a

substrate solution.

The results are shown in Table 2. The results show that the protein recovery percentage is high in the plates of Examples 3 and 4, as compared with in the plate of Comparative Example 2.

Table 2

Protein concentration after storage (comparison of absorbance)

ng/mL	Example 3	Example 4	Comparative Example 2
0.1	0.24	0.26	0.01
1	0.41	0.33	0.07
10	0.76	0.66	0.07
100	1.02	0.82	0.03

(Example 5)

A 2.0 wt/vol% methanol solution of polyhydroxyethyl methacrylate (P-3932, product of SIGMA) (2.5 mL) was injected into a commercially available polystyrene-made tube (Eiken tube for RIA No. 3, 70-12458). Subsequently, the solution was removed from the tube, the tube was inverted so as to prevent the residual solution from remaining at the bottom, and the tube was dried at room temperature for 24 hours, and consequently the surface of the tube was coated with polyhydroxyethyl methacrylate. In the resultant tube, the saturation adsorption amount of proteins is 8.7×10^{-4} pmol/cm^2, and the contact angle between the surface and water is 0°.

(Example 6)

A 0.5 wt/vol% ethanol solution of an MPC polymer (2.5 mL) was injected into a commercially available polystyrene-

made tube (Eiken tube for RIA No. 3, 70-12458), and the tube was allowed to stand at room temperature for 10 minutes. Subsequently, the solution was removed from the tube, the tube was inverted so as to prevent the residual solution from remaining at the bottom, and the tube was dried at room temperature overnight, and consequently the surface of the tube was coated with the MPC polymer. In the resultant tube, the saturation adsorption amount of proteins is 6.5×10^{-4} pmol/cm^2, and the contact angle between the surface and water is 0°.

The MPC polymer was synthesized from an MPC-BMA (butyl methacrylate) copolymer (ratio of MPC to BMA = 3/7) which was prepared according to the procedure described in "Release of a drug from a hydrogel membrane having a structure analogous to that of phospholipid," (Kobunshi Ronbunshu, 46, 591-595 (1989)).

(Comparative Example 3)

A commercially available polystyrene-made tube (Eiken tube for RIA No. 3, 70-12458) was used in "as is" form as a comparative tube.

(Comparison of assay sensitivity)

In order to evaluate the assay sensitivity of a reaction in a solution, the following test was carried out by use of the tubes of Examples 5 and 6 and Comparative Example 3 as reaction containers, and an ELISA ball (amino-group-containing ball, product of Sumitomo Bakelite Co., Ltd.) as a carrier for reaction.

Phosphate buffer (pH 7.4) solutions of biotin hydrazide (product of Dojindo) were prepared in advance (concentration of biotin hydrazide: 0.125 μg/mL, 0.250 μg/mL, and 0.500 μg/mL). By use of the solutions, biotin hydrazide was immobilized onto ELISA balls through covalent bonding via glutaraldehyde, to thereby prepare ELISA balls having three different immobilization densities of biotin hydrazide.

A portion of each ELISA ball at which biotin hydrazide was not immobilized was subjected to blocking by use of skim milk so as to prevent adsorption.

Each of the above-prepared ELISA balls was placed into the tubes of Example 5, Example 6, and Comparative Example 3 (three tubes for each Example), a phosphate buffer (pH 7.4) solution of peroxydase-labeled avidin (product of Cappel) (concentration of avidin: 1 μg/mL) was injected into each tube (500 mL per tube), and reaction was carried out at room temperature for 30 minutes.

After the reaction was completed, non-reacted peroxydase-labeled avidin was washed with a washing solution (phosphate buffer pH 7.4 + 0.05% Tween 20). Subsequently, each ELISA ball was allowed to develop color by use of a commercially available chromophoric kit for peroxidase (ML-1120T, product of Sumitomo Bakelite Co., Ltd.), and then subjected to measurement of absorbance at 450 nm by use of a plate reader.

The results are shown in Table 3. The results show that, in Examples 5 and 6, the absorbance varies linearly with

respect to the density of biotin hydrazide introduced onto the surface of the ELISA ball, and that, in Comparative Example 3, the absorbance does not vary with the different densities of biotin hydrazide.

In Examples 5 and 6, peroxydase-labeled avidin is reacted with only biotin hydrazide introduced onto the surface of the ELISA ball, and thus the absorbance is proportional to the density of biotin hydrazide. In contrast, in Comparative Example 3, peroxydase-labeled avidin remains in the tube due to adsorption, and the remaining avidin may act as a background, to thereby lower assay sensitivity.

Table 3

Biotin-avidin reaction by use of ELISA ball

μg/mL	Example 5	Example 6	Comparative Example 3
0.125	0.22	0.14	1.32
0.25	0.56	0.64	1.36
0.5	1.12	1.27	1.39

(Comparison of protein recovery percentage in containers usable as storage containers)

For comparison of non-specific adsorption, solutions of an enzyme-labeled anti-bovine-albumin antibody (product of Cosmo Bio) were prepared (concentration of the antibody: 0.1 ng/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL); each solution was injected into 24 wells of each plate; the plates were stored at -80°C for 48 hours; and after storage was completed, the concentration of the protein in each solution was measured by use of a substrate solution.

The results are shown in Table 4. The results show that the protein recovery percentage is high in the plates of Examples 5 and 6, as compared with the plate of Comparative Example 3.

Table 4

Protein concentration after storage
(comparison of absorbance)

ng/mL	Example 5	Example 6	Comparative Example 3
0.1	0.18	0.21	0.03
1	0.58	0.78	0.02
10	1.01	1.36	0.03
100	1.63	1.87	0.05

Industrial Applicability

In the container for an immunoassay of the present invention, the adsorption amount of molecules or serum used for the assay is 1×10^{-1} pmol/cm^2 or less, and thus loss of a reagent, which is caused by adsorption, is prevented during storage or dilution of the reagent. Therefore, when the container is used for a liquid-phase reaction, an assay can be carried out at high sensitivity and high accuracy, since there is prevented decrease in reaction efficiency, which is caused by adsorption of molecules to be assayed, or impediment of reaction due to adsorption of unwanted molecules.

When the container is used for a clinical test employing serum, the test can be carried out under conditions similar to those inside the body of an organism, since variation of the structure of serum components, which is caused by adsorption, does not occur in the container.